Data Evaluation Record on the activated sludge respiration inhibition of 1,2-benzisothiazolin-3-one (BIT)

PMRA Submission Number {.....} EPA MRID Number 47759819

Data Requirement: PMRA Data Code:
EPA DP Barcode: 366249
OECD Data Point:
EPA Guideline:

Test material:
Common name: 1,2-Benzisothiazolin-3-one
Chemical name
IUPAC name:
CAS name:
CAS No.: 2634-66-5
Synonyms: BIT
Smiles string:

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CITATION: Schaefer, E.C., and M.E. Matthews. 2006. 1,2-Benzisothiazolin-3-one: An activated sludge, respiration inhibition test. Unpublished study performed by Wildlife International Ltd., Easton, Maryland, and sponsored and submitted by Rohm and Haas Company, Spring House, Pennsylvania. Wildlife International Ltd. Project No.: 129E-120. Rohm and Haas Report No. 06RC-088. The experiment initiation dates were June 5, 2006 (OECD) and June 6, 2006 (EPA); experiment completion date was June 6, 2006 (p. 6). Final report issued August 14, 2006.

EXECUTIVE SUMMARY

This study is acceptable and satisfies the OECD 209/EPA 850.6800 test guideline. The EC50 was 30 mg/L for BIT and the EC50 for 3,5-dichlorophenol was between five and 30 mg/L (12 mg/L). Also, the difference between the two control samples was <15 %.

The effect of BIT (1,2-benzisothiazolin-3-one; PC Code 098901; chemical purity 89.8%) on activated sludge respiration inhibition was studied in activated sludge from Denton, Maryland for 3 hours with aeration at 20 ± 2°C. 3,5-Dichlorophenol was included as the reference substance. The study was conducted in accordance with OECD Guideline for Testing of Chemicals, No. 209, and in compliance with the USEPA, OECD and Japanese Principles of Good Laboratory Practice. The activated sludge was collected from a municipal wastewater treatment plant which receives waste predominantly from domestic sources. After collection, the activated sludge sample was sieved (2 mm screen) and allowed to settle. The supernatant was decanted, and the total suspended solids (TSS) value was determined. Municipal water was added to obtain a TSS value of *ca.* 4000 mg/L. The sludge was mixed with synthetic sewage (at 50 mL/L) and aerated at 20 ± 2°C until use the next day. Prior to use, the pH and TSS values were determined to be 4327 mg/L and 7.8, respectively. The test material was applied directly without formulation; the reference standard was prepared as a solution in NANOpure® water. The synthetic sewage feed was prepared according to OECD Guideline No. 209.

The test system consisted of a 500-mL plastic Erlenmeyer flask prepared with 9.6 mL synthetic sewage feed, 120 mL activated sludge and the appropriate amount of the test substance or reference substance stock solution then diluted to 300 mL with municipal water. The nominal test concentrations of BIT were 1, 3, 10, 30, 100, 300 and 1000 mg/L. The nominal concentrations of the reference substance, 3,5-dichlorophenol, were 3, 15 and 50 mg/L. Two controls were included in the experiment; these controls contained only synthetic sewage feed and municipal water. After preparation, the suspensions were aerated and incubated at 20 ± 2°C for 3 hours. The incubation of the samples was separated by 15-minute intervals; the first and last samples of the sample set were the controls. After 3 hours of incubation, the sample was transferred to a BOD bottle while the dissolved oxygen concentration was monitored every 10 seconds for up to 10 minutes.

The temperature was maintained at 20 ± 2°C throughout the study period (range 20.1-20.6°C). Aeration was performed at a rate sufficient to provide aerobic conditions and maintain solids in suspension (rate not specifically reported). Mass balances were not determined; samples were not analyzed for BIT. Half-lives were not determined. Extractable [^{14}C]residues were not measured. Nonextractable radioactivity was not measured. Volatiles were not collected.

The respiration rate (mg O_2 per liter per hour; oxygen consumption rate) was calculated using the dissolved oxygen (DO) values between 6.5 mg O_2/L and 2.5 mg O_2/L, or over a 10-minute period if DO did not reach *ca.* 2.5 mg O_2/L. Percent respiration inhibition was calculated using the equation from OECD Guideline No. 209. The dose response pattern (percent inhibition

versus test material concentration) was used to calculate the EC50 value. The EC20 and EC80 values were not calculated. The NOEC, the No-observed-effect-concentration, was also not determined.

For BIT (1,2-benzisothiazolin-3-one), the maximum inhibition was 98.4% with a test substance nominal concentration of 1000 mg/L. The EC50 value was determined to be 30 mg/L). For 3,5-dichlorophenol, the EC50 value was determined to be 12 mg/L.

The study authors included graphical representations of the percent inhibition versus concentration for BIT and 3,5-dichlorophenol. The study authors did not include a graph of the inhibition curve.

Results Synopsis for BIT (1,2-benzisothiazolin-3-one):

Test system used: Activated sludge from Denton, Maryland.
Method used: Activated Sludge Respiration Inhibition Test.

Maximum inhibition = 98.4% with a test substance concentration of 1000 mg/L.
EC50 = 30 mg/L
EC20 = 11.5 mg/L
EC80 = 79 mg/L.
NOEC = 1 (one) mg/L.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted in accordance with OECD Guideline 209 and Council of European Communities Directive 67/548/EEC Annex V, Guideline C.11 (p. 1). No significant deviation from the objectives of OECD No. 209 was noted.

COMPLIANCE: This study was conducted in compliance with the USEPA GLP (40 CFR, Part 160; 1989), OECD Principles of Good Laboratory Practice (1998) and Japan MAFF (11 NohSan, Notification No. 6283 (1999), except for some characterization of the reference standard and test material prior to dosing (See Reviewer's Comment #2; p. 3). Signed and dated Data Confidentiality, GLP, Quality Assurance statements, and Report Approval page were provided (pp. 2-5).

A. MATERIALS:

1. Test Material		BIT (1,2-benzisothiazolin-3-one; p. 11).
Chemical Structure:		Not included.
Description:		White solid.
Purity:	Radiochemical purity:	Not applicable.
	Lot No.:	2005-051; Rohm and Haas TD No. 06-011; Wildlife International, Ltd. ID No. 7599.
	Analytical purity:	89.8% (Expiration date January 20, 2010).
	Specific activity:	Not applicable.
	Location of the radiolabel:	Not radiolabeled.
Storage conditions of test chemical:		Ambient conditions (p. 11).
2. Reference Material		3,5-Dichlorophenol (p. 11).
Chemical Structure:		Not included.
Description:		Solid.
Purity:	Radiochemical purity:	Not applicable.
	Lot No.:	15809KI; Wildlife International, Ltd. ID No. 7320.
	Analytical purity:	97.0%.
	Specific activity:	Not applicable.
	Location of the radiolabel:	Not radiolabeled.
Storage conditions of test chemical:		Ambient conditions (p. 11).

Physico-chemical properties of BIT:

Parameter	Value	Comment
Molecular formula	Not reported.	
Molecular weight	Not reported.	
Water solubility	Not reported.	
Vapor pressure/Volatility	Not reported.	
UV Absorption	Not reported.	
Dissociation constant (pKa)	Not reported.	
Partition coefficient (octanol/water) log K_{ow}/K_{ow}	Not reported.	
Stability of compound at room temperature	Not reported.	

3. Activated Sludge Characteristics

Table 1: Description of activated sludge.

Description	Details
Geographic location	The Denton Wastewater Treatment Plant, Denton, Maryland. Sludge from predominately domestic sources.
Pesticide use history at the collection site	Not reported.
Collection date	June 5, 2006.
Collection procedures	Not reported.
Sampling depth	Not reported.
Storage conditions	Not reported.
Storage length	None before processing; 1 day, overnight, after processing.

Data obtained from pp. 12-13 of the study report.

Preparation/Processing: The activated sludge sample was sieved (2 mm screen) and allowed to settle for *ca.* 30 minutes (pp. 12-13). The supernatant was decanted, and the total suspended solids (TSS) value was determined. Municipal water was added to obtain a TSS value of *ca.* 4000 mg/L. The sludge was mixed with synthetic sewage (at 50 mL/L) and aerated at 20 ± 2°C until use the next day. Prior to use, the pH and TSS values were determined.

4. Deionized water and synthetic sewage feed preparation

NANOpure® water was used to prepare the reference stock solution (p. 12).

The synthetic sewage feed was prepared by dissolving the following substances in one liter of municipal water: 16.0 g peptone; 11.0 g meat extract; 3.0 g urea; 0.7 g NaCl; 0.4 g $CaCl_2$ x $2H_2O$; 0.2 g $MgSO_4$ x $7H_2O$; and 2.8 g K_2HPO_4 (p. 12; Appendix II, Appendix I, p. 33).

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None reported.

2. Experimental conditions:

Table 2: Study design.

Parameter		Test substance	Reference substance
Duration of the test		3 hours of incubation.	
Activated sludge condition	Preparation	50 mL of synthetic sewage feed was added to each liter of sludge suspension prior to overnight storage prior to use.	
	pH	7.8, prior to use in test system.	
Mixed liquor suspended solids level (mg/L)		4327 (adjusted from an initial 8000 mg/L after supernatant layer removal).	
Amount of activated sludge per treatment		120 mL.	
Test materials	Stock solutions preparation	No stock solution was prepared; direct addition employed due to low water solubility.	3,5-dichlorophenol (500.1 mg) was dissolved in 10 mL of 1N NaOH solution. The solution was diluted with 30 mL of NANOpure® water, and the pH was adjusted with 10.0 mL of 1N H_2SO_4 solution. The final volume was adjusted to 1L using NANOpure® water.
	Test concentrations (nominal)	1, 3, 10, 30, 100, 300 and 1000 mg/L.	3, 15 and 50 mg/L.
Control conditions, if used		The same as those of the test and reference samples.	
No. of Replications	Controls, if used	Two samples total for study.	
	Treatment	One sample per test concentration was prepared.	
Test apparatus	Type/material/volume	Each 500-mL plastic Erlenmeyer flask was prepared with 9.6 mL synthetic sewage feed, activated sludge and the appropriate amount of the test substance or reference substance stock solution then diluted to 300 mL with municipal water.	
	Details of traps for CO_2 and organic volatiles, if any	Not applicable.	
If no traps were used, is the system closed/open?		Not applicable.	
Identity and concentration of co-solvent		None.	NANOpure® water.
Test material	Volume of the test solution used/treatment:	Not reported.	
	Application method :	Not reported.	
	Is the co-solvent evaporated?	No.	
Any indication of the test material adsorbing to the walls of the test apparatus?		None.	
Microbial biomass of the control (mg microbial carbon)		Not determined.	

Parameter		Test substance	Reference substance
Microbial biomass (mg microbial carbon)		Not determined.	
Experimental conditions:	Temperature (°C):	20 ± 2°C.	
	Continuous darkness:	Not applicable.	
	Aeration:	Aerated at a rate sufficient to provide aerobic conditions and maintain solids in suspension; not further detailed.	
	Intervals between samples:	15 minutes.	
	Moisture content:	Not applicable.	
	Moisture maintenance method:	Not applicable.	
Other details, if any		The first and last samples were the two control samples.	

Data were obtained from pp. 10-15 of the study report.

3. Aerobic/anaerobic conditions: No determinations were made to verify that the aerobic conditions were maintained.

4. Supplementary experiments: None reported.

5. Sampling:

Table 3: Sampling details.

Criteria	Details
Sampling intervals	3 hours of incubation.
Sampling method	The sample was transferred to a BOD bottle (300 mL volume).
Method of collection of CO_2 and organic volatile compounds	Volatiles were not collected.
Sampling intervals/times for:	
Sterility check, if sterile controls are used:	Not applicable.
Moisture content:	Not applicable.
pH levels:	Prior to use in test system.
Redox potential:	Not applicable.
Dissolved oxygen:	The YSI Model 50B Dissolved Oxygen Meter was used to measure dissolved oxygen every 10 seconds during a period of 10 minutes after collection.
Sample storage before analysis	None.
Other observation, if any	

Data were obtained from pp. 12-13 and 15, Appendix I, p. 19 of the study report.

C. ANALYTICAL METHODS:

1. Treated activated sludge:

Extraction/clean up/concentration methods: No extraction/clean up/concentration methods were employed.

Determination of nonextractable residues: Not determined.

Determination of volatile residues: Volatiles were not collected.

Total ^{14}C measurement: Total ^{14}C residues were not determined.

Identification and quantification of parent compound: Samples were not analyzed for BIT.

Detection limits (LOD, LOQ) for the parent compound: Limits of Detection (LOD) and Quantification (LOQ) were not reported.

2. Calculation of results:

The respiration rate (mg O_2 per liter per hour; oxygen consumption rate) was calculated using the dissolved oxygen (DO) values between 6.5 mg O_2/L and 2.5 mg O_2/L, or over a 10-minute period if DO did not reach *ca.* 2.5 mg O_2/L (p. 13). The respiration rate was calculated using the following equation:

Respiration rate = [(initial DO – final DO)/(final time – initial time)] x 3600 s/hr.

In accordance with OECD Guideline No. 209, percent respiration inhibition was calculated using the following equation (pp. 13-14):

Percent inhibition = $\{1 - [2R_s/(R_{c1} + R_{c2})]\} \times 100$

where R_s is the respiration rate at tested concentration of test substance, R_{c1} is the respiration rate of control 1 and R_{c2} is the respiration rate of control 2. All respiration rates were reported in units of mg O_2 L^{-1} h^{-1} (Table 1, p. 17).

The dose response pattern (percent inhibition versus test material concentration) was used to calculate the EC50 value, the concentration of the test substance that reduces the respiration rate by 50% as estimated by the simple first order formation equation (Y=1-a(exp(-bx))). This is equation number 8100 in Tablecurve 2-D. The EC20 and 80 values were 11.5 and 79 mg/L, respectively. The NOEC, the No-observed-effect-concentration, was about 1 mg/L.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: The temperature was maintained at 20 ± 2°C throughout the study period (range 20.1-20.6°C; pp. 12, 14). Aeration was performed at a rate sufficient to provide aerobic conditions and maintain solids in suspension (rate not specifically reported).

B. MATERIAL BALANCE: Material balances were not determined.

C. TRANSFORMATION OF PARENT COMPOUND: The transformation of BIT was not determined.

HALF-LIFE/DT50/DT90: Half-lives were not determined.

EXTRACTABLE RESIUDES: Extractable [^{14}C]residues were not measured.

D. RESPIRATION INHIBITION TEST:

Table 4: Respiration rates and percent inhibition results of the activated sludge respiration inhibition test with BIT (1,2-benzisothiazolin-3-one) and the reference substance (3,5-dichlorophenol).

Test sample	Nominal concentration of substance (mg/L)	Respiration rate (mg O_2 L^{-1} h^{-1})	% inhibition
Control 1	0	36.9	NA
Control 2	0	40.0	NA
3,5-Dichlorophenol	3	27.7	28.0
	15	19.5	49.3
	50	11.6	69.8
BIT	1	52.4	-36.3
	3	33.5	12.9
	10	33.5	12.9
	30	18.3	52.4
	100	6.1	84.1
	300	1.8	95.3
	1000	0.6	98.4

Data were obtained from Table 1, p. 17 of the study report.
NA = Not applicable.

RESULTS: For BIT (1,2-benzisothiazolin-3-one), the maximum inhibition was 98.4% with a test substance nominal concentration of 1000 mg/L (p. 15; Table 1, p. 17). The EC50 value was determined to be 30 mg/L. For 3,5-dichlorophenol, the EC50 value was determined to be 11.5 mg/L).

The study authors included graphical representations of the percent inhibition versus concentration for BIT and 3,5-dichlorophenol (Figures 1-2, p. 18). The study authors did not include a graph of the inhibition curve.

E. SUPPLEMENTARY EXPERIMENT-RESULTS: No supplemental experiments were reported.

III. STUDY DEFICIENCIES

No significant deviations from OECD Guideline No. 209 were noted.

IV. REVIEWER'S COMMENTS

1. The two points of validity criteria of OECD Guideline No. 209 were satisfied by the study (pp. 14-15). The respiration rates of the two controls were 36.9 and 40.0 mg O_2 L^{-1} h^{-1}, respectively, which was a difference of 7.8%. The validity criterion of the OECD Guideline No. 209 is that the difference between the respiration rates of the two controls is 15% or less. The experimentally determined EC50 value of the reference substance 3,5-dichlorophenol was 11.5 mg/L after 3 hours of contact time; the range prescribed by the OECD Guideline No. 209 is 5 to 30 mg/L.

2. The study was performed in accordance with USEPA, OECD and Japanese GLP procedure with the following exceptions: characterization of the reference substance and stability of the reference substance under conditions of storage at the test site were not determined in accordance with GLP (p. 3). Additionally, the concentration, stability and homogeneity of the reference substance and test substance in the carrier were not determined.

3. The preparation of the activated sludge included sieving with a 2 mm mesh (p. 12). OECD Guideline No. 209 does not include sieving in the preparation of the test innoculum (p. 5 of OECD 209). The preparation of the innoculum is described as washing with tap water, if necessary, prior to centrifugation. The innoculum is centrifuged three times to obtain the settled solids for the study. This preparation described by OECD Guideline No. 209 does not specifically eliminate large particles.

4. OECD Guideline No. 209 indicates that a pH-electrode and measuring equipment "is necessary" as equipment for the test (p. 4 of OCED 209). The pH was only taken at the start of the study prior to addition of test material. No measurements of pH were taken during the study at sampling.

V. REFERENCES

1. OECD Guideline for Testing of Chemicals, No. 209, Activated Sludge, Respiration Inhibition Test. 1984.

2. U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, No. 850.6800, Modified Activated Sludge, respiration Inhibition Test for Sparingly Soluble Chemicals. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-96-168.

3. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

4. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.

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